"The colder, the better" seems to be the mantra for structural biology with structures now routinely solved by cooling protein samples to cryogenic temperatures. The improved resolution at low temperatures results from reduced radiation damage by x-rays and electrons to proteins than at room temperature. We have adapted a microfabricated liquid cell to protect liquid protein samples from the vacuum of a conventional TEM. For the first time, we report the ability to image macromolecular assemblies, the horseshoe crab acrosome and bacteriorhodopin, at room temperature and in liquid water. The limit in resolution approaches that reported for these structures in vitreous ice and at liquid nitrogen temperatures. More importantly, we measure the fall-off of the highest intensity reflections from Fourier transforms of electron micrographs or from electron diffraction patterns of the samples and find that radiation damage is reduced not increased in liquid water compared to vitreous ice. We propose that the mechanism of radiation damage differs between the two conditions. Imaging protein in water opens the door to the possibility of studying protein dynamics in real time and to solve structures of protein without freezing.

1968-Pos Board B738

Folding Dynamics and Kinetic Schemes from Signal-Pair Correlation Analysis of Single-Molecule Trajectories

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Kinetic analysis of single-molecule trajectories is typically done by mapping measured signal trajectories (e.g. molecular extension, FRET, or ion current) onto a discrete set of states and then analyzing the dwell times. State identification can be challenging, however, especially in multi-state systems when signals are noisy or have low amplitude. We present a new method which avoids the need to identify states but can still analyze complex systems, based on correlations between "signal pairs," two discrete ranges of the measured signal. The concept is related to a recently-introduced method for analyzing single-molecule FRET measurements of diffusing molecules [1]. First, time correlations are calculated between all signal pairs, without assuming any specific kinetic model for the system. Next, kinetic models are tested to determine the correct scheme, by choosing signal ranges associated with different states and fitting all cross-correlations between them to functions which are derived for each model from previously-described matrix methods [2]. We demonstrate the method with three examples measured by single-molecule force spectroscopy: folding of a two-state DNA hairpin, which can also be analyzed with simple standard methods; a folding of a three-state DNA hairpin, for which the kinetic scheme is known; and folding of the prion protein PrP, which forms non-native structures with an unknown kinetic scheme. These examples show that the method can cope with significant overlap of signals from different states due to noise (as high as 70%), states with very low occupancy (as low as 2%), and transition rates which are very similar or differ by several orders of magnitude. We also introduce signal-pair histograms for an unbiased visualization of dynamic processes within the trajectories.

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1969-Pos Board B739

Pressure Sensitive Reaction of F1-ATPase

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F_1-ATPase is a rotational stepping molecular motor in which γ subunit rotates 120° against the $\alpha_3\beta_3$ cylinder upon one ATP molecule hydrolysis. This 120° step is further divided into 80° and 40° substeps and each substep is triggered by ATP binding and ADP release and by ATP hydrolysis and Pi release, respectively. The stepping motion was sensitive against physical and chemical conditions, such as temperature and load Hydrostatic pressure is also a physical parameter to modulate the structure and function of protein molecules. Here, we developed a novel assay that monitored the stepping motion of single F_1-ATPase molecules under various pressure conditions [1]. At ambient conditions, F_1-ATPases derived from thermophilic *Bacillus* PS3 smoothly rotated with 9 Hz in the presence of 2 mM ATP. The rotational rate decreased with increased pressure, and then reached to 3 Hz and became stepping rotation by slowing a certain dwell at 140 MPa In order to identify which chemical state this dwell corresponds, the mutant F_1(βE190D) which shows the pause of ATP catalytic dwell due to extremely slow ATP hydrolysis even under Vmax condition [2] was used. This pressure dependent dwell became obvious at +40° from catalytic dwell with applying pressure, i.e., it is the same position as ATP binding, where F1 executes ATP binding and ADP release on different catalytic sites. Thus, applied pressures seem to inhibit the ATP binding and/or ADP release reactions.

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1970-Pos Board B740

Single-Image Molecular Analysis for Accelerated Fluorescence Imaging

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We have developed a new single-molecule fluorescence imaging analysis method, SIMA, that improves the temporal resolution of single-molecule localization and tracking studies to millisecond timescales without compromising the nanometer range spatial resolution [1,2,3,4]. In this method, the width of the fluorescence intensity profile of a static or a mobile molecule, imaged using submillisecond to milliseconds exposure time, is used for localization and dynamics analyses. We apply this method to three single-molecule investigations: (1) axial localization precision measurements, (2) subdiffraction molecular separation measurements, and (3) protein diffusion coefficient measurements in free solution. Applications of SIMA in studying intraflagellar transport processes and photosynthetic antenna complex energy transfer mechanisms will also be discussed.

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1971-Pos Board B741

A Hybrid Tirf-Magnetic Tweezers Instrument for Studying Sub-Nanometer Effects of Force on Proteins

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Proteins exert and withstand mechanical force in many fundamental biological processes. Optical tweezers have become a useful research tool for applying forces to single proteins and measuring the resulting changes in extension, but many interesting processes produce changes smaller than their resolution limit. Our experimental setup skirts this limitation by measuring distance changes using single-molecule Förster resonance energy transfer (smFRET) produced from a total internal reflection fluorescence (TIRF) microscope incorporating magnetic tweezers. Individual protein molecules are conjugated to FRET-paired fluorescent dyes and functionalized DNA handles using maleimide and click chemistry. These handles tether each molecule between a glass coverslip within the TIRF microscope and a paramagnetic bead. An external magnet applies a uniform field that exerts a force on each molecule tethered to the surface. Because the FRET from each molecule in the microscope's field of view can be measured simultaneously, the extension between dyes of many individual molecules as a function of force can be monitored in parallel. Here we present our initial studies using this new setup.

1972-Pos Board B742

Simple Method for Stage Drift Correction

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To measure nanometric features with super-resolution requires that the stage that holds the sample be stable to nanometric precision. Herein we introduce a new method which uses conventional equipment, is low cost, and does not require intensive computation. Tiny fiduciary markers of approximately

1um x 1um x 1um in x, y, z dimension are placed at regular intervals on the coverslip. These fiduciary markers are easy to put down, are completely stationary with respect to the coverslip, and are completely bio-compatible. To do this, the fiduciary markers are made by: 1) making a mask out of Si/SiN_3; 2) making a PDMS anti-mask from the silicon mask; 3) placing the PDMS on the coverslip along with commercially-available polymerizing glue; 4) shine UV-light to cause the glue to polymerize and then remove the PDMS. As the coverslip undergoes drift (or is purposely move), the (x, y) center of the fiduciary markers can be easily tracked to 1 nanometer using a Gaussian fit. By focusing the light slightly out-of-focus, the z-axis can also be tracked to <12 nm by looking at the diffraction rings. The process of tracking the fiduciary markers does not interfere with visible fluorescence because an IR-LED (690-850 nm) is used and the IR-light is separately detected using an inexpensive camera. The resulting motion of the coverslip can then be corrected for, either after-the-fact, or using active stabilizers to correct for the motion. We apply this method to watch kinesin walking with $\approx$ 8 nm steps.

1973-Pos Board B743

Super-Resolution Study of the Dynamics and Spatial Distribution of Ribosomes in Live *E. Coli* Cells

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Little is known about the spatial organization and dynamics of the translational machinery in bacteria. We have examined the distribution and diffusion of ribosomes in live *E. coli* cells by localizing and tracking single ribosomes labeled by an S2-eYFP construct expressed from the chromosome. Fast reversible photobleaching of eYFP was used to image single ribosome molecules and obtain the time-averaged spatial distribution of ribosomes with ~30-nm resolution. In medium growth conditions (doubling time 62 minutes) the ribosomes are highly segregated from the nucleoid. In DNA rich regions the concentrations of ribosomes is less than 20% of that in the ribosome-rich regions in the end caps and space between the two nucleoid lobes. This is in reasonable agreement with a Monte Carlo model using realistic parameters for the number of ribosomes and the amount of plectonemic DNA. We have studied dynamics of single ribosomes by using time lapse imaging. The mean diffusion constant is $D_{ribo} = 0.04\ \mu m^2/s$. Halting of transcription with Rifampicin resulted in about 10 fold increase in the diffusion constant and a homogeneous distribution of labels throughout the cytoplasm. The drastic change in dynamics can be explained in part by conversion of polysomes to monomeric ribosomes and 30S subunits in the absence of new mRNA synthesis. Expansion of the nucleoid after rifampicin treatment might be responsible for eliminating ribosome/DNA segregation and may further enhance diffusion. Halting of translation by Chloramphenicol treatment increased the ribosome-DNA segregation as the DNA compacted. The diffusion constant remained the same. Studies of the spatial distribution of different size fluorescent proteins in the presence and absence of drugs suggest that the degree of segregation from DNA is largely size dependent.

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Single-Molecule Fluorescence Meets DNA Origami

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Recent developments in DNA nanotechnology facilitate a new degree of control to place arbitrary objects. Therefore, DNA is folded into arbitrary 2- and 3-dimensional structures on a nanometer to micrometer scale. In this so-called origami technique, introduced by Paul Rothemund in 2006, one ~7.3 kbases long single-stranded DNA is hybridized with ~200 short synthetic DNA ''staple'' strands to build a desired structure by self-assembly. Objects of interest, e.g. single fluorophores, are attached to individual incorporated DNA strands at specific positions within the structure. Several applications of this approach are shown using single-molecule fluorescence techniques.

Revisiting the distance dependence of fluorescence resonance energy transfer (FRET), we used the DNA origami technique to build a spectroscopic ruler. In contrast to double stranded DNA, a commonly used spacer molecule, this technique offers distinct advantages. We designed a rigid DNA origami block, which has a higher persistence length and additionally allows placing the dye molecules all oriented in the same direction on the top surface, limiting static effects of the linker lengths. In contrast to dsDNA, for the origami block the Förster Radius R_0 could directly be obtained from the distance dependence of energy transfer based on single-molecule FRET measurements.

Guided by the idea to build complex spectroscopic networks by self-assembly, we used rectangular DNA origami as a molecular breadboard to precisely position individual fluorophores. In this artificial system the path of energy transfer can be manipulated on the nanoscale. Fluorophores were incorporated such that the light from the ''blue'' input dye could either be guided to the ''red'' or ''IR'' output dye, by a ''green'' dye that was placed at two alternative positions. We used a single-molecule four-color FRET approach with alternating laser excitation for analysis of the different energy transfer paths.

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Establishing the Composition of Alpha-Synuclein Oligomers using Single-Molecule Photobleaching

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Growing evidence suggests that oligomeric aggregation intermediates of the human alpha-synuclein protein are toxic to neurons in Parkinson's disease. Very little is known about the molecular details of these oligomeric aggregates. Conventional techniques to determine the number of monomers per oligomer, such as size exclusion chromatography or mass spectrometry, give estimates ranging from less than 10 to more than 60. It is unclear if this spread is due to influences from the technique used or if it reflects size heterogeneity of the oligomers.

Single-molecule photobleaching allows for direct probing of the number of labeled monomers per oligomer, but is not typically applicable for large oligomers ($>$10 monomers). To overcome this problem, we develop a method that uses sub-stoichiometric labeling, that is, only a fraction of the monomers contain a fluorescent label, in combination with single-molecule photobleaching. The number of bleaching steps now gives the number of fluorescent labels instead of the number of monomers. Via the label probability mass function we link the number of fluorescent labels to the total number of monomers.

Using this approach, we find a single, well defined alpha-synuclein oligomer consisting of 31 monomers. We also demonstrate that the aggregation is stochastic and that there is no influence of the fluorescent label on the aggregation. Fluorescence correlation spectroscopy data indicate a very loose packing of the oligomers. In combination with tryptophan fluorescence measurements, we propose a structural model for the oligomers.